Genetic diversity and genetic differentiation of natural populations of *Pinus kesiya* var. *langbinanensis*

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Abstract: Genetic diversity and genetic differentiation of natural populations of *Pinus kesiya* var. *langbinanensis* were examined by means of electrophoresis technique. Analysis of 9 enzyme systems including 16 loci showed that all the three natural populations of the pine were high in genetic diversity but low in inter-population genetic differentiation. The proportion of polymorphic loci is 0.667, with each locus holding 2.13 alleles, averagely. The average expected and observed heterozygosity was 0.288 and 0.197, respectively. The gene differentiation among populations was 0.052, but the mean genetic distance was only 0.015.
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Introduction

Genetic diversity is the base of biodiversity. For a forest, the richness and pattern of genetic diversity of the tree species are assumed to determine the capability of the underlying forest in adapting the environmental changes, which is a foundation stone in maintaining the stability of a forest ecosystem (Zhang *et al.* 1998). Being predominant in outcross breeding organisms, genetic diversity is the insurance of genetic gain, which has been resulted in a diverse of natural populations in one species. Genetic richness and genetic differentiation of the core populations for one species is one of the most important indexes to evaluate its quality of germplasm resources pool (Zhang *et al.* 2000). So, the genetic diversity investigation plays an important role in realizing the origin and adaptation of species, locating the distribution of gene resources, and planning the utilization of the gene resources effectively and reasonably. One of the quick and easy methods for measuring the genetic diversity is isozyme analysis via starch gel or polyacrylamide gel electrophoresis (Haase 1993).

Szemao pine (*Pinus kesiya* var. *langbinanensis*), distributed mainly in Southeastern Yunnan, is one of the most important timber and resin production tree species in Yunnan Province. Its distribution (Compile Group of Yunnan Forests 1986), ecology (Wu 1994; Wu 1990), biology (Wu *et al.* 1992), and genetic improvement (Zhao *et al.* 1998; Zhao *et al.* 1999) have already been reported. One study reported its karyotypes at the biochemical level (Gu *et al.* 1982). So far, its provenance variation and genetic diversity of natural populations are unknown yet. This study aimed to reveal the genetic diversity and genetic differentiation of three natural populations of Szemao pine through the analysis of enzyme systems, which will provide basic knowledge for the utilization, conservation and improvement of gene resources of Szemao pine.

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Materials and methods

Materials

Three natural populations of Szemao pine with about 20 individuals of each population were sampled from three different places in Southern Yunnan, namely Lanchang county, Jiangcheng county and Jinghong Dadugang of Simao Prefecture. Cones were collected from December of 1999 to January of 2000 in middle part of the crown separately. The seeds from the cones were stored in dry below 0 °C, and endosperms were used in the electrophoresis.

Electrophoresis

From the seeds of each tree, 6-8 endosperms were randomly chosen in the electrophoresis. Since endosperms of conifers are haploids, they can be used directly to test whether the results followed Mendel Rule or not, and to measure locus and to count the number of allele in each locus which displayed in the banding patterns. Enzyme analysis was detected by two electrophoresis, vertical slab polyacrylamide gel electrophoresis and horizontal starch gel electrophoresis. A total of 9 enzyme systems were involved in the examination. Enzyme systems and related electrophoresis methods are listed in Table 1. Details of the electrophoretic procedures were revealed in Chen's (2001) report.

Enzyme phenotype analysis

After staining, gel slices showed isozyme patterns of

various enzyme systems, from which the gene locus and alleles of each locus were read. From negative to positive, the loci were represented by 1, 2, 3 and 4 in turns, and the alleles in each locus by A, B, C and D. Because endosperm is haploidy, the alleles separating in each locus from seeds of the same individual were considered obeying Mendel's Genetic Rule, alleles do not separate from monomorphic loci, thus the separation rate will be 1:1 in polymorphic loci. So, the genotype of individual tree can be read directly from the isozyme patterns and allelic frequencies of each locus can be then calculated.

Table 1. Enzyme systems and related electrophoresis methods of *Pinus kesiya* var. *langbinanensis*

Enzyme systems	Abb.	EC No.	Electrophoresis methods	Extraction buffers	Buffer systems
Aspartate aminotransferase	AAT	E.C.2.6.1.1	Vertical slab polyacrylamide gel	II	
Alcohl dehydrogenase	ADH	E.C.1.1.1.1	Horizontal starch gel	II or III	A or B
Esterase	EST	E.C.3.1.1.-	Vertical slab polyacrylamide gel	I , II or III	
Glutamate-ammonia ligase	GDH	E.C.1.4.1.2	Vertical slab polyacrylamide gel	III	
Glucose-6-phosphate dehydrogenase	G6PD	E.C.1.1.1.49	Horizontal starch gel	II or III	A or B
Malate dehydrogenase	MDH	E.C.1.1.1.37	Horizontal starch gel	III	A
Phosphogluconate dehydrogenase	PGD	E.C.1.1.1.44	Horizontal starch gel	III	A
Phosphoglucomuase	PGM	E.C.5.4.2.2	Horizontal starch gel	III	A
Shikimate dehydrogenase	SKD	E.C.1.1.1.25	Horizontal starch gel	II	A

Notes: Extraction buffer I --Tris-glycin (pH 8.3) + 0.1% mercaptoethanol + 4% PVP; Extraction buffer II --Simple phosphorus acid buffer (0.1M, pH 7.5), (Chen Shaoyu, Zhao Wenshu. 2001); Extraction buffer III--complex phosphorus acid buffer (prepared before use) (Chen Shaoyu, Zhao Wenshu, 2001); Buffer systems A-- Electrode buffer: 0.4M trisodium citrate (pH 7.0); Gel buffer: 0.02M histidine monohydrochloride (pH 7.0); Buffer systems B-- Electrode buffer: 0.1M NaOH, 0.3M boric acid (pH 8.6); Gel buffer: 0.015M Tris, 0.004M citric acid (pH 7.8).

Genetic diversity and genetic differentiation

The following genetic parameters were calculated and analyzed to study genetic diversity and genetic differentiation of natural populations of Szemao pine. They are percentage of polymorphic loci (P); mean number of alleles per locus (A); NEI's unbiased estimate of expected heterozygosity (He), observed heterozygosity (Ho), gene differentiation among populations (G_{ST}), and genetic distances (D).

Results and discussion

Locus and alleles of the 9 enzyme systems

A total of 9 enzyme systems were analyzed in the study (Table 1). Among them, 2 activity zones appear in AAT, namely, 2 loci, Aat-1 and Aat-2. In all three populations, the 2 loci are monomorphic. Locus Adh-2 is not very clear, so Adh-1 and Adh-3 are analyzed, and there are 3 and 4 alleles respectively. There is only 1 locus in GDH with 1-3 alleles in three populations. 2 loci, G6pd-1 and G6pd-2 appear in G6PD, but only G6pd-2 with 2 alleles is analyzed. There are 4 loci in EST, however, only Est-4 is analyzed, which is encoded with alleles of A, B and C. There are also 4 loci in MDH, and 2 alleles in Mdh-1. The other 3 loci are monomorphic. There are 2 loci in PGD, but only Pgd-2 is analyzed, which shows 3 alleles. 3 loci appear in PGM, 4 alleles in Pgm-1, 3 alleles in Pgm-2 and Pgm-3. There are 3 loci in SKD, but only Skd-3 is analyzed, and 2-3 alleles in the locus. So, for all 9-enzyme systems, 16 loci encoded with 38 alleles are taken into genetic analysis.

Genetic diversity of populations

Distribution and frequencies of 38 alleles are listed in Table 2, and indexes of genetic diversity of populations are calculated (Table 3).

From the Table 3, it can be seen that the average number of alleles per locus (A) is 2.13 for all three populations, and of which Dadugang population shares the highest (reaching 2.3). The percentage of polymorphic loci (P) of the three populations is 66.7%, and of which from Dadugang and Lanchang populations hold the same (68.8%), while Jiangcheng population is only 62.5%. The average observed heterozygosity (Ho) of the three populations ranged from 0.173 to 0.219 with a mean of 0.197 and it is similar to the values reported for many other coniferous species (Yeh 1981). Expected heterozygosity (He) values of the three populations range from 0.257 to 0.326 with a mean of 0.288, which is a little higher than the results reported for many other coniferous specie (Hamrick *et al.* 1981). Comparing on the values of heterozygosity of three populations shows that Dadugang population is again the highest, which reaches 0.326 (He) and 0.219 (Ho). It indicates that the genetic diversity of Dadugang population is the richest in the three populations.

Genetic differentiation of populations

Measures of gene diversity provide estimates of population differentiation. Nei (1978) divided the gene diversity (H_T) into two levels: gene diversity within populations (Hs) and gene diversity among populations (D_{ST}). The genetic indexes describing genetic differentiation of Szemao pine populations are given in Table 4. The results show that interpopulation differentiation of Szemao pine is low. The H_T is 0.304 and Hs is 0.288, while D_{ST} is only 0.016. The G_{ST} of populations is 0.052. That is, only 5.2% of the total gene diversity resides interpopulations, while 94.8% of the total gene diversity resides within populations. It is a bit lower than the average level of 15 reported conifer species (G_{ST}=0.061), (Harmrick *et al.* 1989).

Table 2. Allelic frequencies and heterozygosity in three natural populations of *Pinus kesiya* var. *langbinanensis*

Locus	Allele (A, B, C, D) Heterozygosity (He, Ho)	Lanchang	Dadugang	Jiangcheng	Locus	Allele (A, B, C, D) Heterozygosity (He, Ho)	Lanchang	Dadugang	Jiangcheng
	A	1.000	1.000	1.000		A	1.000	1.000	1.000
Aat-1	He	0.000	0.000	0.000	Mdh-2	He	0.000	0.000	0.000
	Ho	0.000	0.000	0.000		Ho	0.000	0.000	0.000
	A	1.000	1.000	1.000		A	1.000	1.000	1.000
Aat-2	He	0.000	0.000	0.000	Mdh-3	He	0.000	0.000	0.000
	Ho	0.000	0.000	0.000		Ho	0.000	0.000	0.000
	A	0.833	0.545	0.929					
	B	0.083	0.273	0.071		A	1.000	1.000	1.000
Adh-1	C	0.083	0.182	0.000	Mdh-4	He	0.000	0.000	0.000
	He	0.292	0.595	0.133		Ho	0.000	0.000	0.000
	Ho	0.333	0.273	0.143					
	A	0.227	0.045	0.143					
	B	0.591	0.727	0.786		A	0.708	0.591	0.808
	C	0.182	0.045	0.071		B	0.125	0.318	0.154
Adh-3	D	0.000	0.182	0.000	Pgd-2	C	0.167	0.091	0.038
	He	0.566	0.434	0.357		He	0.455	0.541	0.322
	Ho	0.273	0.273	0.357		Ho	0.583	0.455	0.385
	A	0.958	0.773	1.000		A	0.208	0.545	0.571
	B	0.042	0.045	0.000		B	0.458	0.273	0.286
Gdh-1	C	0.000	0.182	0.000		C	0.250	0.045	0.071
	He	0.080	0.368	0.000	Pgm-1	D	0.083	0.136	0.071
	Ho	0.083	0.273	0.000		He	0.677	0.607	0.582
						Ho	0.417	0.273	0.214
	A	0.708	0.591	0.346		A	0.833	0.818	0.679
	B	0.292	0.409	0.654		B	0.000	0.000	0.143
Gpd-2	He	0.413	0.483	0.453	Pgm-2	C	0.167	0.182	0.179
	Ho	0.417	0.273	0.231		He	0.278	0.298	0.487
						Ho	0.167	0.364	0.286
	A	0.500	0.227	0.333		A	0.273	0.200	0.462
	B	0.318	0.545	0.542		B	0.591	0.650	0.538
Est-4	C	0.182	0.227	0.125	Pgm-3	C	0.136	0.150	0.000
	He	0.616	0.599	0.580		He	0.558	0.515	0.497
	Ho	0.182	0.364	0.500		Ho	0.364	0.500	0.154
	A	0.000	0.000	0.000		A	0.182	0.227	0.179
	B	0.833	0.818	0.714		B	0.818	0.682	0.821
Mdh-1	C	0.167	0.182	0.286	Skd-3	C	0.000	0.091	0.000
	He	0.278	0.298	0.408		He	0.298	0.475	0.293
	Ho	0.167	0.182	0.286		Ho	0.182	0.273	0.214
Mean	He	0.282	0.326	0.257					
	Ho	0.197	0.219	0.173					

The traits of species, such as geographic range, regional distribution, breeding system, seed dispersal mechanism and successional status, account for great proportion of its genetic diversity and its variance in genetic diversity. Szemao pine tends to have rich genetic diversity because of the traits of long-life, generation overlap, wide distribution, wind pollination and wind- seed dispersal, which lay broad genetic base for the species. On the other hand, distributed in different environment, Szemao pine suffers from the selection presses from nature and human, which lead to the differentiation of populations. Genetic isolation and genetic drift also contribute greatly to the differentiation.

Table 3. Genetic diversity of natural populations of *Pinus kesiya* var. *langbinanensis*

Populations	Average no. of alleles per locus (A)	Percentage of polymorphic loci (P)	Mean heterozygosity	
			Observed (Ho)	Expected (He)
Lanchang	2.1	68.8	0.198	0.282
Dadugang	2 3	68 8	0 219	0 326
Jiangcheng	2 0	62 5	0.173	0.257
Mean	2.13	66.7	0.197	0.288

Table 4. Genetic differentiation of *Pinus kesiya* var. *langbinanensis* natural populations

Locus	H_T	H_S	D_{ST}	G_{ST}
Aat-1	0.000	0.000	0.000	0.000
Aat-2	0.000	0.000	0.000	0.000
Adh-1	0.381	0.340	0.041	0.107
Adh-3	0.475	0.452	0.023	0.048
Gdh-1	0.167	0.149	0.018	0.108
Gpd-2	0.491	0.450	0.041	0.083
Est-4	0.624	0.598	0.026	0.042
Mdh-1	0.334	0.328	0.006	0.018
Mdh-2	0.000	0.000	0.000	0.000
Mdh-3	0.000	0.000	0.000	0.000
Mdh-4	0.000	0.000	0.000	0.000
Pgd-2	0.458	0.439	0.019	0.000
Pgm-1	0.665	0.622	0.043	0.064
Pgm-2	0.364	0.354	0.010	0.027
Pgm-3	0.543	0.523	0.020	0.037
Skd-3	0.363	0.355	0.008	0.022
Mean	0.304	0.288	0.016	0.052

Note: H_T ---Total gene diversity; H_S ---Gene diversity within populations; D_{ST} --Gene diversity among populations; G_{ST} Gene differentiation among populations

The degree of genetic differentiation is analyzed by using Nei's genetic distance (Nei 1978). In Table 5, the genetic distance and genetic identity of the populations of Szemao pine are presented (Above diagonal: genetic identity; below diagonal: genetic distance). The average genetic distance is 0.015.

Table 5. Genetic identity and distance among the populations of *Pinus kesiya* var. *langbinanensis*

Populations	1	2	3
1. Nuofuxiang	-	0.987	0.983
2. Dadugng	0.013	-	0.985
3. Jiangcheng	0.018	0.015	-

Notes. above diagonal. genetic identity, below diagonal genetic distance

Conclusions

Enzyme electrophoresis is an economical and very useful technique for analysis of genetic diversity. Here, Szemao pine was studied on 9 enzyme systems with 16 loci by two combined electrophoresis methods. Through genetic analysis on 16 loci encoded with 38 alleles, the index of genetic diversity and differentiation of populations were calculated. The results show that the genetic diversity of the three natural populations of Szemao pine is rich. The values of A, P, He and Ho are a bit higher than those of other Conifer species. Genetic differentiation between populations (G_{ST}) is a bit lower than the average level of 15 reported conifer species. The total genetic diversity of populations is 0.304, but only 5.2% of which comes from inter-populations. The average genetic distance of populations is 0.015.

Verification of genetic diversity and genetic distinctness of regional or local tree stands as potential genetic resources. Szemao pine is common and very important tree species of Yunnan Province and may become more important for the forestry development in the future. The study may be helpful for the further development and utilization of the tree resources.

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